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THE VIBRATILE ORAL MEMBRANES OF GLAUCOMA SCINTILLANS, EHR.

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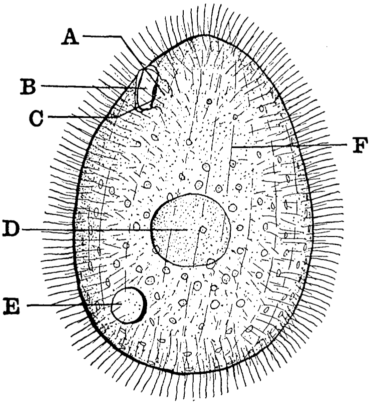
Glaucoma scintillans was first described by Ehrenberg in 1838, and has received since that time but little notice, save as a species to be included in catalogues of microfauna. The writer has found it of interest because of the peculiar appendages borne about the mouth, *i.e.,* the vibratile oral membranes. Other species also bear either one or two oral membranes, but *Glaucoma* is distinct in this respect: that the membranes completely encircle the oral aperture; are distinctly bilabial in form, and are usually in active, characteristic motion. So rapid is the motion of the membranes that, due to the reflection and refraction of the light rays from the microscope mirror which is produced, a twinkling or scintillating appearance results. It is from this phenomenon that the creature has received its specific name, *scintillans.*

The body of *Glaucoma* is ovate (Figs. 1 and 2), the ventral surface flattened and entirely ciliated; the dorsal surface arched, and naked. The cuticle is everywhere longitudinally striated. The oral aperture lies in the anterior half of the ventral surface, its longer axis slightly oblique, though in many individuals the longer oral axis is practically parallel with the longer axis of the body. The nucleus is spherical, its diameter being approximately equal to the long axis of the mouth, and situated in the central portion of the body, often, however, slightly nearer the posterior extremity. A secondary nucleus, roughly a fifth or sixth of the diameter of the primary one, can frequently be made out. The contractile vacuole is usually single, rarely double, and situated in the posterior third of the body. In shape, *Glaucoma* is more constant than many of the other ciliates. The adults are usually almost perfectly oval or ovate; the young are apt to be often spherical. In size the adults vary between 70 and 75 microns in length. The writer has noted a few individuals that were 78–80



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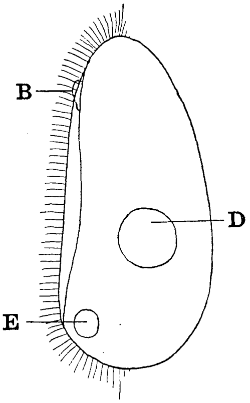

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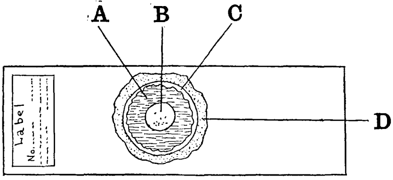

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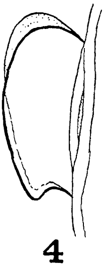

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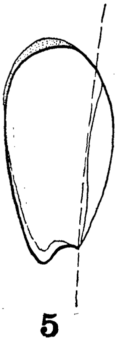

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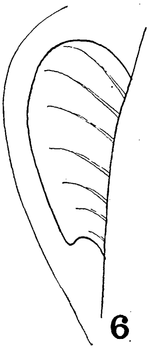

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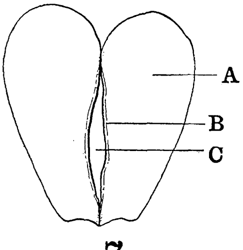

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microns.[1] These, however, were very rare, and did not number more than perhaps a dozen individuals among several hundred.

Glaucoma is a fairly common protozoan. Its usual habitat is in stagnant water, particularly pond water in which there is considerable decaying aquatic vegetation. The writer has found it abundant in the brackish water of a peat swamp. It commonly occurs wherever there are also numbers of small flagellates. Samuelson ('57)[2] says that he has reared large numbers of them in an infusion made from cabbage leaves and distilled water. Cultures of *Glaucoma* were prepared in the laboratory by the writer by allowing water, containing cattails, ponds lily leaves, and *Elodea* to become putrid. Numerous *Glaucomæ* were found congregated in the grayish scum that overspread the surface of the infusions, where they were engaged in feeding upon the bacteria, which seems to form the bulk of their food.

It was necessary to keep individual *Glaucomæ* under observation over extended periods of time, and this was accomplished by means of a device whose utility was such as to warrant a description of it here (Fig. 3). A piece of lens paper, or of thin typewriter manifolding paper,

FIG. 1. Mature *Glaucoma scintillans*, ventral aspect. *A*, indefinite gullet; *B*, vibratile oral membranes or outer lips; *C*, opening to buccal cavity (or mouth proper), or inner lips; *D*, nucleus; *E*, contractile vacuole; *F*, striations of the cuticle.

FIG. 2. Outline drawing of the lateral aspect of *Glaucoma scintillans*. *B*, outer lips; *D*, nucleus; *E*, contractile vacuole. Note that the ventral surface may often be depressed.

FIG. 3. Micro-aquarium. *A*, circle of thin lens paper, bearing a circular opening; *B*, *C*, the cover glass: *D*, sealing material (parafin or Canada balsam).

FIG. 4. The vibratile oral membranes, or outer lips, with the opening of the mouth just beneath them. Semi-diagrammatic.

FIG. 5. The outer lips dissociated from their connection with the cuticle. The broken line represents the position of the surface of the cuticle.

FIG. 6. A single outer lip, showing the transverse striations.

FIG. 7. The outer lips represented as being spread out flat, and seen from above. *A*, outer lips; *B*, inner lips; *C*, opening of mouth proper.

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[1] Eyferth (1909) gives the length of *Glaucoma scintillans* as lying between the extremes, 20 and 80 microns! The lesser figure probably refers to the length of the young, just after division.

[2] *Quart. Jour. Mic. Sci.*, Vol. 5, 1857, p. 19.

the shape and size of the cover glass, with a small circular opening cut in its center, is soaked in hot parafin, drained, and placed upon a glass slide. After the parafin has cooled a drop of water containing the organisms is placed in the center of the circular opening, and the cover glass applied. The drop should flatten out just sufficiently to fill completely the space between the cover glass and the slide inside the circular opening. The cover glass must now be sealed down about its edge by means of a camel's hair brush dipped in hot paraffine. Such a device the writer has called a *micro-aquarium* and has found its employment of the utmost use. In it scores of *Glaucomæ* were kept alive for as long as eight hours.

For quieting the movements of *Glaucomæ* thick gelatine solution was used, and a drop, mixed with a drop of water containing the organisms, placed on a slide and examined under the 16 mm. objective and allowed to remain uncovered until evaporation had rendered the mixture of such viscosity as to retard the animals sufficiently. The cover glass was then applied and sealed down with either hot paraffine or castor oil to prevent further evaporation.

For killing without staining and without distortion either a one per cent. aqueous solution of copper sulphate, a ten per cent. solution of alcohol, or a fifteen per cent. solution of chloretone was found satisfactory. The best of all killing reagents for *Glaucoma,* however, was found to be a two per cent. aqueous solution of alum.

Intra-vitam staining was accomplished with an aqueous solution of Bismarck and methyl green; while for staining after killing there were utilized: iodine, methyl green, methyl blue, Bismarck brown, and safranin.

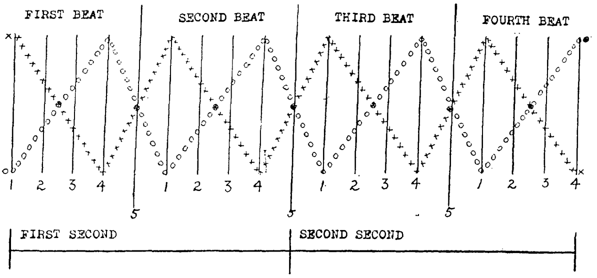
The appendages which make *Glaucoma* of especial interest are the oral membranes. These are present in the form of two hyaline, lip-like structures, lying one on either side of the buccal cvaity (Fig. 1). For convenience these will be referred to as the lips. In form they are roughly rectangular, and approximately twice as long as broad, with one extremity enlarged and rounded, and the other slightly narrowed and with a crescentric edge (Fig. 4). They are joined at their base, at either end, and project outward from the body so as to be plainly visible in profile (Fig. 2). Usually the lips are in constant motion, but occasionally may be seen inactive, and when so are normally closed (Fig. 1).

When the body of *Glaucoma* is disintegrated with strong alum solution, or copper sulphate, it is sometimes possible to isolate the two lips together, they apparently being more resistant than the rest of the body. When this is done, it can be seen that they are more nearly oval in outline, possessing an extended, fin-like portion, which projects into, or beneath the cuticle (Figs. 4 and 5).

That the origin of these membranes may be sought for in the fusion of rows of cilia situated on either side of the buccal cavity, is suggested by the fact that each lip exhibits striations running at right angles, or nearly so, to the longitudinal axis (Fig. 6). These striations are broadest at their bases, and narrowest at their tips, and recall to mind the rows of stout oral cilia found among so many of the infusoria.

Between the bases of the lips lies the opening of the buccal cavity, or mouth proper. Its edges, which may be termed the inner lips, are also capable of a motion which will be described later (Fig. 7).

The movement of the outer lips consists of a rapid opening and closing, and is best described by the word, beating. The rate of the beating is apparently under control of the animal, and varies at different times. Under normal conditions the beating of the lips is very rapid. The hyaline membranes, even when at rest, seem to glisten, possibly due to the striations which refract the light in different ways, and when in rapid motion produce the pretty scintillating effect which has given the creature its name. Observations made by the writer of the rate of opening and closing of the lips, showed that it varies from eight or nine times per second, through all the lessening degrees, to actual cessation. Usually the beating, no matter what the rate, is regular, *i.e.,* the durations of opening and closing being of equal length. This, however, is subject to modification, apparently at will, and individuals are sometimes, though not frequently, encountered in which the lips beat with great



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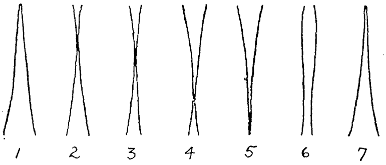

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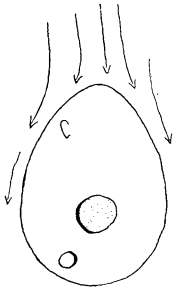

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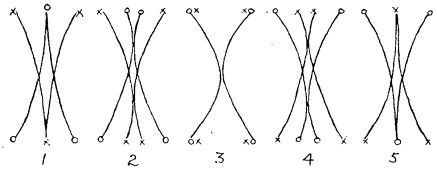

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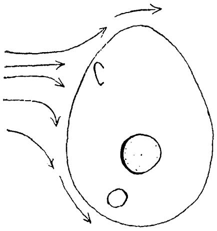

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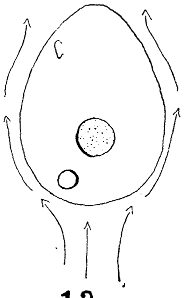

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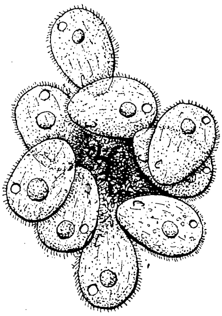

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rapidity for a few seconds, then flutter slowly, then close for a second or two, and then begin slowly to beat again. From experiments which have been made the conclusion is drawn that the rate of the beating of the oral membranes in *Glaucoma* is directly proportional to the strength of the food stimulus received in the buccal cavity, or possibly through the cilia in the region of the mouth.

The lips do not open and close with their ectal edges parallel. The process of closing takes place with a wave-like motion, the anterior edges of the lips meeting first, and the wave of motion thus initiated traveling toward the posterior ends, until, when these finally meet, the anterior edges are open again. The process of opening, therefore, takes place in the reverse direction. Fig. 8 diagrams six stages in a single beat of the lips, and in Fig. 9 are represented four beats analyzed with respect to their cycles of contacts and partings.

The opening and closing of the inner lips, or edges of the mouth proper, take place, apparently, in a similar wave-like manner, and in the reverse order or motion from that of the outer lips. This sequence of motion may aid in forcing food into the buccal cavity, since whenever any given portion of the buccal cavity is open

FIG. 8. Six stages in a cycle of the opening and closing of the outer lips. The edges of the lips are represented diagrammatically to show how the waves of contact and parting follow each other. Stage 7 begins the cycle anew.

FIG. 9. Graphic representation of the relations of the cycles of opening and closing of the outer lips. Four beats are analyzed, each of one half second's duration. The vertical lines represent the line of the outer edges of the lips, and the lines of crosses and circles show how the waves of contact and parting sweep up and down them. Four stages are given for each beat—represented by the four vertical lines of the lips in each beat. The longer vertical lines represent the stages when no part of the lips are in contact. Stages 1, 2, 3, 4, 5, in this figure correspond respectively to stages 1, 2, 4, 5, 6, in Fig. 8.

FIG. 10. Five stages of the cycles of opening and closing of both the outer and inner lips diagrammatically represented, and superimposed to show their reverse relationships. The *o*-lines represent the edges of the outer lips, and the *x* lines the edges of the inner lips.

FIGS. 11, 12, and 13 show the various directions from which water currents, bearing food, can be urged towards an individual by the action of the cilia.

FIG. 14. Group of *Glaucomæ* clustered about a mass of debris and bacteria, feeding.

to receive food, the corresponding portion of the lips above is closed, to force it in. Fig. 10 shows diagrammatically the relationship of the cycles of opening and closing of the outer and inner lips. It will be noted that once during each cycle (at its middle) both the outer and inner lips correspond, for an instant, in attitude, the same portions of each being in contact (Stage 3, Fig. 10). In some individuals the inner lips seemed not to open and close at all, but appear to remain permanently open. Observations on the motions of the lips were made while the animals were incarcerated in very thick gelatine, and all of their movements much retarded.

The ciliary action of the anterior portion of the body can apparently be regulated according to the desires of the animal and water currents, bearing food, can be drawn toward the mouth either from directly in front of the creature, from the side, or from behind (Figs. 11, 12, and 13).

Glaucoma feeds upon minute flagellates and other minute protozoans, fragments of débris, tiny diatoms and desmids, but the bulk of its food seems to consist of bacteria. Fig. 14 shows what may frequently be observed in a culture, *i.e.*, a group of individuals clustered about a mass composed mainly of bacteria, pressing their lips against the substance, and urging portions of it into the buccal cavity by the combined action of the lips, and oral cilia. When feeding in this fashion the movements of the outer lips resembles somewhat the biting and chewing motions of the mouths of the higher animals.

Curiously enough the complex development of the exterior oral appendages seems to have no parallel within the buccal cavity, since neither supporting pharyngeal rods, nor even any definite pharynx, could be discerned.